T-cell regulation: **Thy-1 – hiding in full view**

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Thy-1 is a highly abundant glycoprotein on the surface of thymocytes and neurons. A null mutation in the gene encoding Thy-1 deregulates T-cell receptor signaling and causes abnormal thymocyte development; does this mean that Thy-1 has a signaling function?

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Current Biology 1997, 7:R774–R777
http://biomednet.com/elecref/09609822007R0774

Cellular responses have to be regulated not only positively, so that they are activated in response to appropriate stimuli, but also negatively, to ensure that the response has appropriate magnitude and time course. Such negative regulation may be especially important in the case of dynamic multicellular systems such as the immune system, which is characterized by life-long self-renewal, latent hyperproliferative capability and the potential for fatal autoaggression. Recent years have seen considerable progress in uncovering some of the molecular mediators that contribute to the negative regulation of the immune system. For instance, studies on inhibitory receptors expressed by natural killer cells have revealed the crucial roles that they play in limiting inappropriate cytotoxicity [1].

Negative regulatory functions have also been ascribed to a variety of molecules expressed by B and T lymphocytes. For B cells, these include the cell surface glycoprotein CD22, the intracellular Src-related tyrosine kinase Lyn, and the protein tyrosine phosphatase Shp-1 [2]. The inhibitory actions of these molecules impinge on the sensitivity of signaling through the B-cell antigen receptor, and thereby influence B-cell responses and tolerance. For T cells, the CTLA-4 glycoprotein mediates an essential negative regulatory function, as made dramatically clear by the fatal lymphoproliferative disorder of CTLA-4 null mutant mice [3]. Several other studies with knockout mice have suggested that a number of cell surface molecules found on T cells may have negative regulatory functions [4–8]. One of these molecules is the ever-enigmatic Thy-1 (CD90).

Since Thy-1 was first identified serologically in the 1960s [9], it has been the subject of research that has led to several fundamental discoveries. Firstly, antisera that were specific for Thy-1 were the first reagents that could discriminate T cells from B cells [10], an attribute of profound experimental significance that needs little elaboration. Secondly, structural studies on Thy-1 in the 1970s [11] helped to establish the foundation of the immunoglobulin superfamily, the known members of which now comprise approximately one third of all known leukocyte membrane proteins [12]. Thirdly, the structural analysis of Thy-1 also led to the first biochemical description of a vertebrate glycosyl phosphatidylinositol (GPI) anchor [13,14]. Such anchors are now known to provide a general mechanism for tethering a wide range of surface proteins to the plasma membrane. Thus, as a subject of experimentation, Thy-1 has proven to be remarkably rewarding over the course of three decades. Paradoxically, despite this lengthy and rich history, Thy-1's own function has remained elusive.

Thy-1 is a small heavily glycosylated glycoprotein made up of a single immmunoglobulin-related domain, to which three *N*-linked carbohydrates are attached. Although its expression pattern shows some variability between species, Thy-1 is found on T cells, thymocytes and neurons in mice. In the thymus, Thy-1 is probably the most abundant glycoprotein on the surface of thymocytes and is present at about a million molecules per cell (compare this to ~15,000 molecules of CD4 per cell). Indeed, by one calculation, Thy-1 may cover 10–20% of the thymocyte surface area [12]. Thus, Thy-1 is perhaps well described as a major architectural component of the thymocyte surface, a distinction that could be intimately tied to its normal function.

As a possible clue to the function of Thy-1, considerable interest has surrounded the signaling effects of Thy-1-specific antibodies [15]. In large part, this is because of the apparent contradiction of transmembrane signaling by a molecule that does not pass through the inner leaflet of the plasma membrane. Interestingly, GPI-linked molecules such as Thy-1 cosegregate with protein tyrosine kinases in detergent-insoluble complexes of cellular lysates [16]. This cosegregation may reflect the regulated (perhaps ligand-directed) formation of physiologic associations that would allow Thy-1 to transmit signals. However, the natural formation of such associations — in the absence of exogenously applied antibody — has yet to be demonstrated, leaving in doubt the potential relevance of the antibody-dependent signaling effects. Furthermore, no ligand for Thy-1 has yet been defined in firm molecular terms.

To pursue the function of Thy-1 further, null mutant mice bearing a disruption of the Thy-1 gene were recently generated. The initial description of these animals focused entirely on the nature of a unique neuronal defect

[17]. Specifically, the loss of Thy-1 correlated with regional impairment of long-term potentiation in the dentate gyrus of the hippocampus. Despite this defect, Thy-1-deficient brains were anatomically normal, and the mice showed no detectable problems with spatial learning. A subsequent report on the immunologic phenotype of the Thy-1 mutant mice was published recently in *Current Biology* [7]. At first glance, the Thy-1 mutation appears to have an unexpectedly modest effect on T cells; however, a careful examination of the mutant thymocytes uncovered evidence of a signaling defect that is curiously reminiscent of observations made on several null mutant animals lacking other T-cell surface molecules [4–6,8].

The essence of the defect in Thy-1-null thymocytes appears not to be inefficient signaling, but an enhanced response to mitogenic stimulation through the T-cell antigen receptor. This is manifest at the level of tyrosine phosphorylation, calcium mobilization and proliferation. Interestingly, there are developmental abnormalities that accompany the signaling phenotype and that could well be a direct consequence of it. Specifically, there is impaired development of mature thymocytes expressing the CD8 coreceptor, particularly in Thy-1 mutants that also carry a T-cell receptor transgene. A kinetic analysis revealed further evidence for abnormal development, apparent as a reduced rate of production of mature 'single positive' cells — CD4 or CD8 T cells — and a possible decreased survival of their 'double-positive' (CD4, CD8) precursors. Finally, these same double-positive thymocytes appear to be enriched for cells that retain expression of *RAG-1* — required for the joining of germ-line gene segments to form a productive T-cell receptor gene — a property indicative of an atypical immature status and consistent with the view that the absence of Thy-1 causes a developmental barrier.

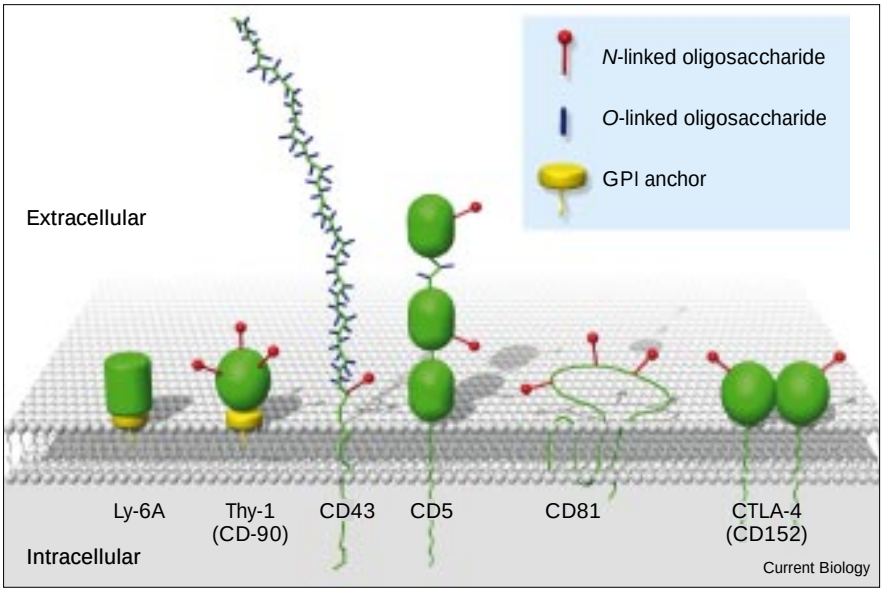
Enhanced signaling through the T-cell receptor is perhaps not an obvious cause of inefficient development. One possible explanation for the apparent correlation is that the phenotype may in fact be one of exaggerated negative selection. In this scenario, more thymocytes would be deleted when Thy-1 is absent, because strong T-cell receptor signaling is likely to increase the fraction of cells that would undergo apoptosis. Interestingly, CD5-null mice exhibit a similar signaling defect and have developmental problems that are made particularly clear when the mutant mice also carry a T-cell receptor transgene [4]. But while the Thy-1 mutation causes impaired development of T-cell receptor transgenic CD8 T cells, the CD5 mutation has the opposite effect. The basis for this apparent discrepancy is unclear, but it could reflect quantitative differences in the way that the Thy-1 and CD5 mutations affect T-cell receptor signaling. Specifically, the lack of CD5 could result in a weaker derepression of signaling than the lack of Thy-1, so that the selection of cells expressing the transgenic T-cell receptor is potentiated by the former, whereas apoptosis results from the latter.

Alternatively, rather than the loss of negative regulation, the developmental barrier in the Thy-1-null thymus could be due to the absence of a positive function of Thy-1 that is necessary for efficient development. This last idea prompts further consideration of how the absence of Thy-1 might influence T-cell receptor signaling and affect thymocyte development. As one possible approach to this, it may be useful to consider the related hyper-responsive phenotypes caused by deficiencies of several other cell surface molecules (see Figure 1).

Of the five molecules shown in Figure 1, the negative regulatory effect conferred by CD43 may be the most straightforward to understand. This molecule is highly expressed on T cells and thymocytes, and it is also a scaffold for extensive negative charge in the form of *O*-linked glycosylation and sialylation [12]. As a consequence of its abundance and negative charge, CD43 is well equipped to interfere with the formation of intercellular conjugates. Consistent with this, the absence of CD43 causes spontaneous aggregation of T cells, presumably because of a loss of electrostatic repulsion between the cells [8,18]. Thus, an enhanced capacity to form conjugates could well result in more frequent or productive engagements of T-cell receptors and costimulatory molecules; this in turn could account in large part for the observed signaling phenotype exhibited by CD43-null T cells.

CD5 and CD81, in contrast, may be expected to have a more direct impact on antigen receptor signaling. These two molecules can be coimmunoprecipitated with key signal transduction modules found on the surface of T cells and B cells, respectively. CD5 associates with components of the T-cell antigen receptor [19], and CD81 is linked to the CD19/CD21 complex [20]. Although the significance of the CD5–T-cell receptor association is uncertain, stimulation of the T-cell receptor leads to the rapid tyrosine phosphorylation of an ITAM-like motif in the cytoplasmic tail of CD5 [21]. Perhaps more significantly, there is one report of an association between CD5 and SHP-1 [22], and another showing a similar association with PI-3 kinase [23], both of which are intracellular molecules with negative regulatory functions. The CD81/CD19/CD21 complex provides a potent costimulatory function for B-cell activation and it too undergoes rapid phosphorylation after surface immunoglobulin crosslinking [20]. Interestingly, CD81 was first identified with a monoclonal antibody that interfered with the proliferation of lymphoblastoid cells [24]. Thus, for both CD5 and CD81, there is an existing framework of signaling data within which to consider possible mechanisms of negative regulation.

Figure 1



T-cell surface molecules implicated in the negative regulation of T-cell receptor signaling and antigen-specific proliferation. (Not to scale.)

Thy-1 and Ly-6A are both GPI-linked structures. As mentioned above, this endows them with *in vitro* signaling properties that may have physiologic significance. Such signaling properties could be involved in negative regulation of antigen receptor signaling, perhaps through the formation of plasma membrane microdomains that would sequester signaling molecules such as protein tyrosine kinases. However, it is also possible that the absence of Thy-1 may have a more indirect effect on the efficiency of signaling, along the lines of the mechanism proposed above for CD43.

As Thy-1 normally occupies a large fraction of the thymocyte surface, its absence should be expected to have a profound effect on the physical appearance and behaviour of the cell surface. This may be apparent either in the way that key molecules are distributed on the surface of thymocytes, or alternatively in the way that thymocytes interact with other cells such as thymic epithelial cells or medullary macrophages. Interestingly, electron microscopy of the Thy-1-null thymus revealed striking evidence of atypical contacts between cells, consistent with the idea that intercellular interactions are abnormal because of the mutation. Exactly how such abnormal intercellular interactions would translate into augmented responses to T-cell receptor stimulation is not immediately apparent.

A ligand for Thy-1, if it exists, could allow for firm conjugation with the thymic stroma and may thereby facilitate the transduction of signals that would influence the responsiveness of the T-cell receptor. A precedent for this is well established in the form of interactions between CD4 and class II major histocompatibility complex molecules that downregulate T-cell receptor expression on double-positive thymocytes [25]. Alternatively, the Thy-1-depleted surface may allow for more effective clustering of T-cell receptors, perhaps because they are localized in a different fashion on the cell surface or because there is less material — like Thy-1-attached oligosaccharides — to impede their aggregation. Whatever its precise molecular basis, the key issue here may be the highly abundant expression of Thy-1, which could indirectly affect T-cell receptor signal transduction in a way that does not necessarily involve a unique signaling role for Thy-1 itself.

Clearly, the function of Thy-1 still remains elusive, despite the interesting phenotype of the Thy-1 knockout mouse. An important challenge for the future may lie in understanding precisely how Thy-1 influences the physical characteristics of the cell surface and the exact nature of its effect on T-cell receptor signaling. In this regard, Thy-1-null thymocytes may be especially useful experimentally and one cannot help but suspect that lessons of fundamental cell biological importance may still be forthcoming from this unusual molecule.

Acknowledgements

I thank Jason Cyster, Debbie Law, Art Weiss and Linda Zuckerman for their comments on the manuscript.

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